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L4: Entry 8 of 9 File: USPT May 22, 2001

DOCUMENT-IDENTIFIER: US 6235514 B1
TITLE: Nucleic acid molecules encoding isopentenyl monophosphate kinase, and methods of use

Abstract Text (1):
A cDNA encoding isopentenyl monophosphate kinase (IPK) from peppermint (Mentha x piperita) has been isolated and sequenced, and the corresponding amino acid sequence has been determined. Accordingly, an isolated DNA sequence (SEQ ID NO:1) is provided which codes for the expression of isopentenyl monophosphate kinase (SEQ ID NO:2), from peppermint (Mentha x piperita). In other aspects, replicable recombinant cloning vehicles are provided which code for isopentenyl monophosphate kinase, or for a base sequence sufficiently complementary to at least a portion of isopentenyl monophosphate kinase DNA or RNA to enable hybridization therewith. In yet other aspects, modified host cells are provided that have been transformed, transfected, infected and/or injected with a recombinant cloning vehicle and/or DNA sequence encoding isopentenyl monophosphate kinase. Thus, systems and methods are provided for the recombinant expression of the aforementioned recombinant isopentenyl monophosphate kinase that may be used to facilitate its production, isolation and purification in significant amounts. Recombinant isopentenyl monophosphate kinase may be used to obtain expression or enhanced expression of isopentenyl monophosphate kinase in plants in order to enhance the production of isopentenyl monophosphate kinase, or isoprenoids derived therefrom, or may be otherwise employed for the regulation or expression of isopentenyl monophosphate kinase, or the production of its products.

Brief Summary Text (4):
Isopentenyl diphosphate (IPP) is the central intermediate in the biosynthesis of isoprenoids in all organisms. In higher plants, the formation of IPP is compartmentalized. The mevalonate (MVA) pathway, the enzymes of which are localized to the cytosolic compartment, produces the precursor of triterpenes (sterols) and certain sesquiterpenes (Newman, J. D. & Chappell, J., Crit. Rev. Biochem. Mol. Biol., 34:95-106 [1999]). In plastids, the deoxyxylulose-5-phosphate (DXP) pathway operates to supply IPP for the synthesis of monoterpenes and diterpenes (Eisenreich, W. et al., Tetrahedron Lett., 38:3889-3892 [1997]; Eisenreich, W. et al., Proc. Natl. Acad. Sci. USA, 93:6431-6436 [1996]), several sequiterpenes (McCaskill, D. & Croteau, R., Planta, 197:49-56 [1995]), tetraterpenes (carotenoids), and the prenyl side-chains of chlorophyll and plastoquinone (Lichtenthaler, H. K. et al., FEBS Lett., 400:271-274 [1997]).

Brief Summary Text (5):
In addition, there are examples of cooperation between the cytosolic and plastidial pathways in the biosynthesis of stress-induced and constitutively emitted volatile terpenoids from a variety of plants (Piel, J. et al., Angew. Chem. Int. Ed., 37:2478-2481 [1998]), and constitutive sesquiterpenes of chamomile (Adam, K.-P. & Zapp, J., Phytochemistry, 48:953-959 [1998]). In mammals, where the DXP pathway is not known to operate, and in plants, the individual biosynthetic steps of the MVA pathway have been well-characterized (Goldstein, J. L. & Brown, M. S., Nature (London), 343:425-430 [1990]; Bach, T. J., Crit. Rev. Biochem. Mol. Biol., 34:107-122 [1999]). However, for the recently discovered DXP pathway, which also occurs in many eubacteria (Rohmer, M., Prog. Drug Res., 50:135-154 [1998]), the biosynthetic sequence leading to the formation of IPP is still incompletely defined (The FIGURE).

Brief Summary Text (6):
The initial step of the pathway involves a condensation of pyruvate (C2 and C3) with D-glyceraldehyde-3-phosphate (GAP) to yield 1-deoxy-D-xylulose-5-phosphate (Rohmer, M., Biochem. J., 295:517-524 [1993]; Broers, S. T. J., Ph.D. thesis, Eidgenossische Technische Hochschule, Zurich, Switzerland [1994]; Schwarz, M. K., Ph.D. thesis,

Eidgenossische Technische Hochschule, Zurich, Switzerland [1994]; Rohmer, M. et al., J. Am. Chem. Soc., 118:2564-2566 [1996]). The enzyme which catalyzes this reaction belongs to a novel family of transketolases, and the corresponding gene has been isolated from Escherichia coli (Sprenger, G. A. et al., Proc. Natl. Acad. Sci. USA, 94:12857-12862 [1997]; Lois, L. M. et al., Proc. Natl. Acad. Sci. USA, 95:2105-2110 [1997]), peppermint (Lange, B. M. et al., Proc. Natl. Acad. Sci. USA, 95:2100-2104 [1998]) and pepper (Bouvier, F. et al., Plant Physiol., 117:1423-1431 [1998]). In the second step of this pathway, rearrangement and reduction of DXP yield 2-C-methyl-D-erythritol (MEP) (Duvold, T. et al., Tetrahedron Lett., 38:4769-4772 [1997]; Duvold, T. et al., Tetrahedron Lett., 38:6181-6184 [1997]; Sagner, S. et al., Tetrahedron Lett., 39:2091-2094 [1998]) (The FIGURE). Recently, genes encoding this DXP reductoisomerase (DXR) have been cloned from E. coli (Takahashi, S. et al., Proc. Natl. Acad. Sci. USA, 95:9879-9884 [1998]), peppermint (Lange, B. M. & Croteau R., Arch. Biochem. Biophys., 365:170-174 [1999]), and Arabidopsis thaliana (Lange, B. M. & Croteau R., Arch. Biochem. Biophys., 365:170-174 [1999]; Schwender, J. et al., FEBS Lett., 455:140-144 [1999]). To date, no other intermediates on the route to IPP, the terminal product of the DXP pathway (McCaskill, D. & Croteau R., Tetrahedron Lett., 40:653-656 [1999]; Arigoni, D. et al., Proc. Natl. Acad. Sci. USA, 96:1309-1314 [1999]), have been identified.

Brief Summary Text (7):
As disclosed herein, sequencing of 1300 anonymous clones (expressed sequence tags, ESTs) from a cDNA library constructed from mRNA isolated from the oil gland secretory cells of peppermint (Mentha x piperita) (McCaskill, D. & Croteau, R., Planta, 197:49-56 [1995]), afforded, after extensive database comparisons, two clones having homologues of unknown function in plants and eubacteria, the sequences of which contained a motif with homology to the putative ATP-binding domain of the GHMP (galactokinase, homoserine kinase, mevalonate kinase, and phosphomevalonate kinase) family of metabolite kinases. This putative kinase gene from peppermint and its E. coli orthologue, when overexpressed in E. coli, yielded a recombinant enzyme that catalyzes the ATP-dependent phosphorylation of isopentenol monophosphate (IP) to IPP. Feeding experiments with IP and several other isoprenoid precursors, using isolated peppermint secretory cells, confirmed the phosphorylation of IP to IPP to be the last step in the DXP pathway.

Brief Summary Text (9):
In accordance with the foregoing, a cDNA encoding isopentenyl monophosphate kinase (IPK) from peppermint (Mentha x piperita) has been isolated and sequenced, and the corresponding amino acid sequence has been deduced. Accordingly, the present invention relates to isolated DNA sequences which code for the expression of isopentenyl monophosphate kinase, such as the sequence designated SEQ ID NO:1 which encodes an isopentenyl monophosphate kinase protein (SEQ ID NO:2) from peppermint (Mentha x piperita). Additionally, the present invention relates to isolated, recombinant isopentenyl monophosphate kinase proteins, such as the isolated, recombinant isopentenyl monophosphate kinase protein from peppermint (Mentha x piperita) (SEQ ID NO:2). In other aspects, the present invention is directed to replicable recombinant cloning vehicles comprising a nucleic acid sequence, e.g., a DNA sequence which codes for an isopentenyl monophosphate kinase, or for a base sequence sufficiently complementary to at least a portion of DNA or RNA encoding isopentenyl monophosphate kinase to enable hybridization therewith (e.g., antisense RNA or fragments of DNA complementary to a portion of DNA or RNA molecules encoding isopentenyl monophosphate kinase which are useful as polymerase chain reaction primers or as probes for isopentenyl monophosphate kinase or related genes). In yet other aspects of the invention, modified host cells are provided that have been transformed, transfected, infected and/or injected with a recombinant cloning vehicle and/or DNA sequence of the invention. Thus, the present invention provides for the recombinant expression of isopentenyl monophosphate kinase, and the inventive concepts may be used to facilitate the production, isolation and purification of significant quantities of recombinant isopentenyl monophosphate kinase (or of its primary enzyme products) for subsequent use, to obtain expression or enhanced expression of isopentenyl monophosphate kinase in plants, microorganisms or animals, or may be otherwise employed in an environment where the regulation or expression of isopentenyl monophosphate kinase is desired for the production of this kinase, or its enzyme product, or derivatives thereof.

Detailed Description Text (9):

The term "essential oil plant," or "essential oil plants," refers to a group of plant species that produce high levels of monoterpenoid and/or sesquiterpenoid and/or diterpenoid oils, and/or high levels of monoterpenoid and/or sesquiterpenoid and/or diterpenoid resins. The foregoing oils and/or resins account for greater than about 0.005% of the fresh weight of an essential oil plant that produces them. The essential oils and/or resins are more fully described, for example, in E. Guenther, The Essential Oils, Vols. I-VI, R. E. Krieger Publishing Co., Huntington N.Y., 1975, incorporated herein by reference. The essential oil plants include, but are not limited to:

Detailed Description Text (13):
Rutaceae (e.g., citrus plants); Rosaceae (e.g., roses); Myrtaceae (e.g., eucalyptus, Melaleuca); the Gramineae (e.g., Cymbopogon (citronella)); Geranaceae (Geranium) and certain conifers including Abies (e.g., Canadian balsam), Cedrus (cedar), Thuja, Pinus (pines) and Juniperus.

Detailed Description Text (14):
The range of essential oil plants is more fully set forth in E. Guenther, The Essential Oils, Vols. I-VI, R. E. Krieger Publishing Co., Huntington N.Y., 1975, which is incorporated herein by reference.

Detailed Description Text (15):
The term "angiosperm" refers to a class of plants that produce seeds that are enclosed in an ovary.

Detailed Description Text (16):
The term "gymnosperm" refers to a class of plants that produce seeds that are not enclosed in an ovary.

Detailed Description Text (25):
The terms "transformed host cell," "transformed" and "transformation" refer to the introduction of DNA into a cell. The cell is termed a "host cell", and it may be a prokaryotic or a eukaryotic cell. Typical prokaryotic host cells include various strains of E. coli. Typical eukaryotic host cells are plant cells, such as maize cells, yeast cells, insect cells or animal cells. The introduced DNA is usually in the form of a vector containing an inserted piece of DNA. The introduced DNA sequence may be from the same species as the host cell or from a different species from the host cell, or it may be a hybrid DNA sequence, containing some foreign DNA and some DNA derived from the host species.

Detailed Description Text (37):
The gene, or other nucleic acid molecule, encoding isopentenyl monophosphate kinase may be incorporated into any organism (intact plant, animal, microbe), or cell culture derived therefrom, that produces isopentenol monophosphate and ATP to effect the ATP-dependent conversion of these primary substrates to isopentenol diphosphate and its subsequent metabolic products, depending on the organism. By way of non-limiting example, an isopentenyl monophosphate kinase gene (or other nucleic acid molecule encoding isopentenyl monophosphate kinase) may be introduced into a plant in order to increase flux through the isoprenoid biosynthetic pathway that produces carotenoids, chlorophyll, plastoquinone, essential oils, resins, phytoalexins (such as casbene). The resulting transgenic plants can be selected for such improved characteristics as: improved plant fitness, improved defense capabilities against pests and pathogens, improved quality traits (such as color, flavor, vitamin content, antioxidants, nutrients and nutraceuticals) and improved yield of useful chemicals (such as pigments, vitamins, essential oils, resins, waxes and synthetic intermediates). Moreover, and by way of non-limiting example, a nucleic acid molecule encoding an isopentenyl monophosphate kinase protein can be subjected to mutagenesis in order to create isopentenyl monophosphate kinase mutant proteins that are resistant to isopentenyl monophosphate kinase-specific herbicides. Additionally, the isolated, recombinant isopentenyl monophosphate kinase proteins of the present invention can be used, for example, in studies to identify novel antibiotics, herbicides and anti-malarial drugs directed to isopentenyl monophosphate kinase.

Detailed Description Text (42):
Cell cultures derived from multicellular organisms, such as plants, may be used as

hosts to practice this invention. Transgenic plants can be obtained, for example, by transferring plasmids that encode isopentenyl monophosphate kinase and a selectable marker gene, e.g., the kan gene encoding resistance to kanamycin, into Agrobacterium tumifaciens containing a helper Ti plasmid as described in Hoeckema et al., Nature 303:179-181 [1983] and culturing the Agrobacterium cells with leaf slices of the plant to be transformed as described by An et al., Plant Physiology 81:301-305 [1986]. Transformation of cultured plant host cells is normally accomplished through Agrobacterium tumifaciens, as described above. Cultures of mammalian host cells and other host cells that do not have rigid cell membrane barriers are usually transformed using the calcium phosphate method as originally described by Graham and Van der Eb (Virology 52:546 [1978]) and modified as described in sections 16.32-16.37 of Sambrook et al., supra. However, other methods for introducing DNA into cells such as Polybrene (Kawai and Nishizawa, Mol. Cell. Biol. 4:1172 [1984]), protoplast fusion (Schaffner, Proc. Natl. Acad. Sci. USA 77:2163 [1980]), electroporation (Neumann et al., EMBO J. 1:841 [1982]), and direct microinjection into nuclei (Capecchi, Cell 22:479 [1980]) may also be used. Additionally, animal transformation strategies are reviewed in Monastersky G. M. and Robl, J. M., Strategies in Transgenic Animal Science, ASM Press, Washington, D.C., 1995. Transformed plant calli may be selected through the selectable marker by growing the cells on a medium containing, e.g., kanamycin, and appropriate amounts of phytohormone such as naphthalene acetic acid and benzyladenine for callus and shoot induction. The plant cells may then be regenerated and the resulting plants transferred to soil using techniques well known to those skilled in the art.

Detailed Description Text (43):
In addition, a gene regulating isopentenyl monophosphate kinase production can be incorporated into the plant along with a necessary promoter which is inducible. In the practice of this embodiment of the invention, a promoter that only responds to a specific external or internal stimulus is fused to the target cDNA. Thus, the gene will not be transcribed except in response to the specific stimulus. As long as the gene is not being transcribed, its gene product is not produced.

Detailed Description Text (44):
An illustrative example of a responsive promoter system that can be used in the practice of this invention is the glutathione-S-transferase (GST) system in maize. GSTs are a family of enzymes that can detoxify a number of hydrophobic electrophilic compounds that often are used as pre-emergent herbicides (Weigand et al., Plant Molecular Biology 7:235-243 [1986]). Studies have shown that the GSTs are directly involved in causing this enhanced herbicide tolerance. This action is primarily mediated through a specific 1.1 kb mRNA transcription product. In short, maize has a naturally occurring quiescent gene already present that can respond to external stimuli and that can be induced to produce a gene product. This gene has previously been identified and cloned. Thus, in one embodiment of this invention, the promoter is removed from the GST responsive gene and attached to a isopentenyl monophosphate kinase gene that previously has had its native promoter removed. This engineered gene is the combination of a promoter that responds to an external chemical stimulus and a gene responsible for successful production of isopentenyl monophosphate kinase.

Detailed Description Text (45):
In addition to the methods described above, several methods are known in the art for transferring cloned DNA into a wide variety of plant species, including gymnosperms, angiosperms, monocots and dicots (see, e.g., Glick and Thompson, eds., Methods in Plant Molecular Biology, CRC Press, Boca Raton, Fla. [1993], incorporated by reference herein). Representative examples include electroporation-facilitated DNA uptake by protoplasts in which an electrical pulse transiently permeabilizes cell membranes, permitting the uptake of a variety of biological molecules, including recombinant DNA (Rhodes et al., Science, 240:204-207 [1988]); treatment of protoplasts with polyethylene glycol (Lyznik et al., Plant Molecular Biology, 13:151-161 [1989]); and bombardment of cells with DNA-laden microprojectiles which are propelled by explosive force or compressed gas to penetrate the cell wall (Klein et al., Plant Physiol. 91:440-444 [1989] and Boynton et al., Science, 240:1534-1538 [1988]). Transformation of Taxus species can be achieved, for example, by employing the methods set forth in Han et al., Plant Science, 95:187-196 [1994], incorporated by reference herein. A method that has been applied to Rye plants (Secale cereale) is to directly inject plasmid DNA, including a selectable marker gene, into developing floral tillers (de la Pena et al., Nature 325:274-276 [1987]). Further, plant viruses can be used as vectors

to transfer genes to plant cells. Examples of plant viruses that can be used as vectors to transform plants include the Cauliflower Mosaic Virus (Brisson et al., Nature 310:511-514 [1984]). Additionally, plant transformation strategies and techniques are reviewed in Birch, R. G., Ann Rev Plant Phys Plant Mol Biol, 48:297 [1997]; Forester et al., Exp. Agric., 33:15-33 [1997]. The aforementioned publications disclosing plant transformation techniques are incorporated herein by reference, and minor variations make these technologies applicable to a broad range of plant species.

Detailed Description Text (46):
Each of these techniques has advantages and disadvantages. In each of the techniques, DNA from a plasmid is genetically engineered such that it contains not only the gene of interest, but also selectable and screenable marker genes. A selectable marker gene is used to select only those cells that have integrated copies of the plasmid (the construction is such that the gene of interest and the selectable and screenable genes are transferred as a unit). The screenable gene provides another check for the successful culturing of only those cells carrying the genes of interest. A commonly used selectable marker gene is neomycin phosphotransferase II (NPT II). This gene conveys resistance to kanamycin, a compound that can be added directly to the growth media on which the cells grow. Plant cells are normally susceptible to kanamycin and, as a result, die. The presence of the NPT II gene overcomes the effects of the kanamycin and each cell with this gene remains viable. Another selectable marker gene which can be employed in the practice of this invention is the gene which confers resistance to the herbicide glufosinate (Basta). A screenable gene commonly used is the .beta.-glucuronidase gene (GUS). The presence of this gene is characterized using a histochemical reaction in which a sample of putatively transformed cells is treated with a GUS assay solution. After an appropriate incubation, the cells containing the GUS gene turn blue. Preferably, the plasmid will contain both selectable and screenable marker genes.

Detailed Description Text (47):
The plasmid containing one or more of these genes is introduced into either plant protoplasts or callus cells by any of the previously mentioned techniques. If the marker gene is a selectable gene, only those cells that have incorporated the DNA package survive under selection with the appropriate phytotoxic agent. Once the appropriate cells are identified and propagated, plants are regenerated. Progeny from the transformed plants must be tested to insure that the DNA package has been successfully integrated into the plant genome.

Detailed Description Text (58):
The isopentenyl monophosphate kinase protein having the sequence set forth in SEQ ID NO:2 includes a putative amino terminal membrane insertion sequence at residues 1 through 98, and in the embodiment shown in SEQ ID NO:2 directs the enzyme to plastids. Alternative trafficking sequences from plants, animals and microbes can be employed in the practice of the invention to direct the gene product to the cytoplasm, endoplasmic reticulum, mitochondria or other cellular components, or to target the protein for export to the medium. These considerations apply to the overexpression of isopentenyl monophosphate kinase, and to direction of expression within cells or intact organisms to permit gene product function in any desired location.

Detailed Description Text (69):
Bacterial Strains and Plasmid Constructs: A .lambda.ZAP cDNA library was constructed from mRNA obtained (Logemann, J., Schell, J. & Willmitzer, L., Anal. Biochem., 163:16-20 [1987]) from isolated peppermint oil gland secretory cells (McCaskill, D. & Croteau, R., Planta, 197:49-56 [1995]) according to the manufacturer's instructions (Stratagene). Randomly picked and purified clones were excised in vivo and inserts of the resulting pBluescript SK.sup.31 phagemids were partially sequenced from both ends. An apparently full-length, peppermint IP kinase clone (designated ml100)(SEQ ID NO:1) was acquired by this means and was used as a template to amplify by PCR the portion of the sequence of SEQ ID NO:1 extending from residue 3 to residue 1217, using the primers 5'-ATGGCTTCCTCCT-CCCATTTCCTC-3' (forward) (SEQ ID NO:3) and 5'-TTCAGCATCCTGAGGAAAAGACGG-3' (reverse) (SEQ ID NO:4), which was subsequently cloned into the expression vector pBAD TOPO TA (Invitrogen). E. coli strain BL21-CodonPlus-RIL (F.sup.31 ompT, hsdS(r.sub.B.sup.- m.sub.B.sup.-), dcm.sup.30, Tet.sup.R, gal, endA, Hte, [argU, ileY, leuW, Cam.sup.R]; Invitrogen) served as host

in the transformation. The putative E. coli IP kinase gene (SEQ ID NO:5) was amplified by PCR using the primers 5'-ATGCGGACACAGTGGCCCTC-3' (forward) (SEQ ID NO:7) and 5'-AAGCATGGCTCTGTGCAATG-3' (reverse) (SEQ ID NO:8), and genomic DNA from the strain K-12 MG1655 (wild-type) as a template. For expression, the amplicon was cloned into pBAD TOPO TA (Invitrogen) and transformed into E. coli strain TOP10 One Shot (F.sup.-, mcrA, .DELTA.(mrr-hsdRMS-mcrBC) .phi.80lacZ.DELTA.M15, .DELTA.lacX74, recA1, deoR, araD139 .DELTA.(ara-leu)7697, galU, galK, rpsL, (Str.sup.R), endA1, nupG; Invitrogen).

Detailed Description Text (72):
Isolation of and Feeding Studies with Peppermint Oil Gland Secretory Cells: Leaves (15-20 g; <10 mm in length) were excised from peppermint plants (Mentha x piperita L. cv. Black Mitcham) and the oil gland secretory cells were isolated by the glass bead abrasion method (McCaskill, D. & Croteau, R., Planta, 197:49-56 [1995]). Following isolation, the secretory cells were washed with 25 mM Tris/HCl buffer (pH 7.3) containing 200 mM sorbitol, 10 mM sucrose, 5 mM MgCl.sub.2, 10 mM KCl, 1 mM ethyleneglycol bis(.beta.-aminoethyl ether), 8.5 mM Na.sub.2 HPO.sub.4, and 0.1 mM Na.sub.4 P.sub.2 O.sub.7, and then suspended in the same buffer supplemented with 2 mM ATP, 0.1 mM NADPH, 0.1 mM NAD.sup.+, 5 mM phosphoenol pyruvate, and 5 mM glucose-6-phosphate. Cell density was determined using a hemocytometer and was adjusted to 1-2.times.10.sup.5 cellular disks (each containing eight secretory cells) per milliliter suspension. Aliquots (1-1.5 ml) were transferred to 15 ml screw-cap glass vials, and the suspended cells were aerated and incubated at 23.degree. C. for 2 h. At the end of the incubation period, the suspension was extracted three times with 1 ml diethyl ether. The combined organic extract was washed with 1 ml of 1 M Na.sub.2 CO.sub.3 and dried over Na.sub.2 SO.sub.4. An aliquot was removed for liquid scintillation counting and, to the remainder, authentic standards (10-50 .mu.g each) of isopentenol, dimethylallyl alcohol, geraniol, farnesol, limonene, menthone, menthol, pulegone, humulene, and caryophyllene were added. These extracts were then slowly concentrated on ice under a gentle stream of N.sub.2 to .about.200 .mu.l, and were then transferred to conical glass vials and further concentrated to 5-10 .mu.l at 20.degree. C. in preparation for chromatographic analysis.

Detailed Description Text (76):
The oil glands (glandular trichomes) of mint species are highly specialized for the production of monoterpenes and sesquiterpenes, and the secretory cells of these structures are thus highly enriched in the machinery for terpenoid biosynthesis (Lange, B. M. & Croteau, R., Curr. Opin. Plant Biol., 2:139-144 [1999]). As described in Example 1, 1300 random clones obtained from an enriched cDNA library, constructed specifically from mRNA isolated from peppermint glandular trichome secretory cells as described in Example 1 herein, were analyzed. Since the most advanced, defined intermediate of the plastidial DXP pathway to isoprenoids is 2-C-methyl-D-erythritol-4-phosphate (Duvold, T., Bravo, J. M., Pale-Grosdemange, C. & Rohmer, M., Tetrahedron Lett., 38:4769-4772 [1997]; Duvold, T., Cali, P., Bravo, J. M. & Rohmer M., Tetrahedron Lett., 38:6181-6184 [1997]; Sagner, S., Eisenreich, W., Fellermeier, M., Latzel, C., Bacher, A. & Zenk, M. H., Tetrahedron Lett. 39:2091-2094 [1998]), and the end product of the pathway is IPP (McCaskill, D. & Croteau R., Tetrahedron Lett., 40:653-656 [1999]; Arigoni, D., Eisenreich, W., Latzel, C., Sagner, S., Radykewicz, T., Zenk, M. H. & Bacher, A, Proc. Natl. Acad. Sci. USA, 96:1309-1314 [1999]), a phosphorylation step must occur at some point during this reaction sequence. Accordingly, metabolite phosphokinases were sought, but only two clones with similarity to adenylate kinases were noted by searching the common databases. However, a more detailed search of the Prosite database (http://www.expasy.ch/prosite) revealed another more promising clone (designated ml100) (SEQ ID NO:1) which shared a region of high sequence similarity to the putative ATP-binding domain of the GHMP family of kinases (Tsay, Y. H. & Robinson, G. W., Mol. Cell. Biol., 11:620-631 [1991]). The deduced amino acid sequence of this peppermint clone (SEQ ID NO:2) additionally showed significant homology to a chromoplast-directed protein of unknown function from ripening tomato fruits (Lawrence, S. D. Cline, K. & Moore, G. A., Plant Mol. Biol., 33:483-492 [1997]) and to a number of hypothetical proteins from several eubacteria.

Detailed Description Text (84):
The peppermint IPK gene (SEQ ID NO:1) contains an open reading frame of 1218 nucleotides. The first 98 deduced amino acid residues display the general characteristics of plastidial targeting sequences (von Heijne, G., Steppuhn, J. &

Herrmann, R. G., Eur. J Biochem., 180:535-545 [1989]), and, when this putative leader peptide is excluded, a mature protein of 308 amino acids with a predicted size of about 33 kDa is obtained. The gene encoding E. coli IPK (SEQ ID NO:5) consists of 852 nucleotides, which corresponds to an enzyme of 283 amino acids with a size of 31 kDa (SEQ ID NO:6). Database sequence comparison of translated, putative IPK genes from several different organisms revealed very high similarity/identity scores within the plant kingdom (>81.6/74.8% for presumptive orthologues found in tomato (SEQ ID NO:9 encoding the protein of SEQ ID NO:10) and Arabidopsis thaliana (SEQ ID NO:11 encoding the protein of SEQ ID NO:12)), and a high degree of sequence variation among eubacteria (39.0-70.2/25.6-62.5%) and between plants and eubacteria (38.3-48.8/28.5-38.6%). The isopentenyl diphosphate kinases appear to share a conserved, glycine-rich sequence motif (PXGAGLGGGSSNAAX.sub.(15-16) (K/R) (SEQ ID NO:13) similar to the conserved sequence PXXXGL(G/S)SS(A/G)XX.sub.(12-25) (K/R) (SEQ ID NO:14) found in the GHMP family of kinases, including galactokinase, homoserine kinase, mevalonate kinase and phosphomevalonate kinase (Tsay, Y. H. & Robinson, G. W., Mol. Cell. Biol., 11:620-631 [1991]). A related motif is also present in protein kinases (Hanks, S. K., Quinn, A. M. & Hunter, T., Science, 241:42-52 [1988]). The gene for the A. thaliana IPK orthologue is located on chromosome 2 (AC005168; BAC F12C20; PID g3426035), near the marker B68, and contains 10 introns. Neither the intron/exon organization nor a phylogenetic analysis reveals a direct evolutionary relationship between different classes of the GHMP kinase family (data not shown). A detailed survey of the available microbial genome project databases did not indicate the IPK gene (SEQ ID NO:1) to be part of a cluster with other (potential) genes of the DXP pathway.

Detailed Description Text (87):
Although IP was shown to be the preferred substrate of both peppermint IPK (SEQ ID NO:2) and E. coli IPK (SEQ ID NO:6), it remained to be directly demonstrated that IP was an intermediate of the DXP pathway. Previous experiments with isolated peppermint oil gland secretory cells had demonstrated that the MVA pathway in these cells is blocked at an early stage, and that IPP utilized for both monoterpene and sesquiterpene biosynthesis is synthesized exclusively in the plastids from pyruvate (McCaskill, D. & Croteau, R., Planta, 197:49-56 [1995]), almost certainly via the DXP pathway (Eisenreich, W. et al., Tetrahedron Lett., 38:3889-3892 [1997]). The high degree of metabolic specialization and the ability to synthesize monoterpenes and sesquiterpenes de novo from basic precursors, including phosphorylated intermediates (McCaskill, D. & Croteau, R., Planta, 197:49-56 [1995]), made the isolated secretory cells an ideal model system to establish that IP was an intermediate of the DXP pathway, and the activity of IP kinase, in vivo.

Detailed Description Text (89):
Since the IP kinase (SEQ ID NO:2) is plastidial, as is monoterpene biosynthesis, whereas sesquiterpene biosynthesis is cytosolic (McCaskill, D. & Croteau, R., Planta, 197:49-56 [1995]), uptake and partitioning differences between the C.sub.5 precursors influence the distribution between monoterpene and sesquiterpene biosynthetic pathways. In a similar fashion, endogenous phosphatases of both plastidial and cytosolic origin can complicate the partitioning of precursors into the pathways of these compartments. Thus, as a measure of the conversion efficiency of each C.sub.5 precursor, total monoterpenoids (C.sub.10) and sesquiterpenoids (C.sub.15), including geraniol and farnesol released by phosphatases from the corresponding diphosphate ester intermediates, were recorded. By this measure, IPP was most readily converted to terpenoid end-products as expected (561 pmol (h 10.sup.5 cell clusters).sup.-1), followed by ISO (304 pmol (h 10.sup.5 cell clusters).sup.-1), most likely reflecting efficient plastidial uptake of this low molecular weight alcohol, and then IP (43 pmol (h 10.sup.5 cell clusters).sup.-1). DMAPP and DMAP were not very efficient precursors of terpenoids in secretory cells (<6 pmol (h 10.sup.5 cell clusters).sup.-1), and the incorporation of DMA was negligible. Although ISO, likely because of uptake rates, and the more advanced precursor IPP were transformed to terpenoids in vivo at higher rates than was IP, the latter was incorporated at a rate (43 pmol (h 10.sup.5 cell clusters).sup.-1) comparable to that observed previously with pyruvate (67 pmol (h 10.sup.5 cell clusters).sup.-1) (McCaskill, D. & Croteau, R., Planta, 197:49-56 [1995]), an efficient, established precursor of the DXP pathway.

Other Reference Publication (4):
Bouvier, F. et al., "Dedicated Roles of Plastid Transketolases during the Early Onset

of Isoprenoid Biogenesis in Pepper Fruit," Plant Physiol., 117:1423-1431 (1998).

Other Reference Publication (5):
Lawrence, S.D., et al., "Chromoplast development in ripening tomato fruit: identification of cDNAs for chromoplast-targeted proteins and characterization of a cDNA encoding a plastid-localized low-molecular-weight heat shock protein," Plant Mol. Biol., 33:483-492 (1997).

CLAIMS:

4. An isolated nucleic acid molecule of claim 3 encoding a eukaryotic plant isopentenyl monophosphate kinase.

7. An isolated nucleic acid molecule of claim 6 encoding an essential oil plant isopentenyl monophosphate kinase.

14. A replicable expression vector of claim 13 comprising a nucleic acid sequence encoding a plant isopentenyl monophosphate kinase.

18. The method of claim 17 wherein said host cell is a plant cell.

09/988,863

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 * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

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                                                           ENTRY    SESSION
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=> s erg8
L1          27 ERG8

=> s l1 and kinase and activit###
L2           6 L1 AND KINASE AND ACTIVIT###

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3           3 DUP REM L2 (3 DUPLICATES REMOVED)

=> d l3 1-3 bib ab kwic

L3   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2003 ACS on STN
AN   2002:391285  CAPLUS
DN   136:381391
TI   Phosphomevalonate kinase genes from plants identified by
     sequence homology and their use in screening for herbicides
IN   Meissner, Ruth; Lechelt-Kunze, Christa
PA   Bayer AG, Germany
SO   Ger. Offen., 18 pp.
     CODEN: GWXXBX
DT   Patent
LA   German
FAN.CNT 1
     PATENT NO.         KIND  DATE           APPLICATION NO.   DATE
     ---------------    ----  --------       ---------------   --------
PI   DE 10057755         A1   20020523       DE 2000-10057755  20001122
     EP 1209236          A1   20020529       EP 2001-126453    20011109
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
     JP 2002355067       A2   20021210       JP 2001-350270    20011115
     US 2002123427       A1   20020905       US 2001-988863    20011121
PRAI DE 2000-10057755  A    20001122
AB   Plant genes showing sequence homol. to the phosphomevalonate
     kinase gene ERG8 of Saccharomyces cerevisiae are
     identified for use in the development of herbicides acting on isoprenoid
     biosynthesis.  The Arabidopsis thaliana phosphomevalonate kinase
     gene was identified by suppression subtractive hybridization.
TI   Phosphomevalonate kinase genes from plants identified by
     sequence homology and their use in screening for herbicides
AB   Plant genes showing sequence homol. to the phosphomevalonate
     kinase gene ERG8 of Saccharomyces cerevisiae are
```

identified for use in the development of herbicides acting on isoprenoid biosynthesis. The Arabidopsis thaliana phosphomevalonate **kinase** gene was identified by suppression subtractive hybridization.

ST phosphomevalonate **kinase** gene discovery plant herbicide development

IT Protein sequences
(for phosphomevalonate **kinase** sequence homolog of Arabidopsis; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT cDNA sequences
(for phosphomevalonate **kinase** sequence homologs of plants; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT Gene, plant
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(for phosphomevalonate **kinase**, identification by sequence homol. of; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT Genetic methods
(gene discovery; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT Hormones, plant
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(phosphomevalonate **kinase** and biosynthesis and **activity** of; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT Herbicides
Molecular cloning
(phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT Arabidopsis thaliana
Cotton
Medicago truncatula
Pine (Pinus radiata)
(phosphomevalonate **kinase** sequence homolog of; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT Antibodies
RL: AGR (Agricultural use); ARG (Analytical reagent use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(to phosphomevalonate **kinase** sequence homologs; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT 427909-05-5
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT 199693-91-9, GenBank AA660847 234641-71-5, GenBank AI727861 427909-04-4 427909-06-6
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT 9026-46-4, Phosphomevalonate **kinase**
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(sequence homologs; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

IT 427909-20-4
RL: PRP (Properties)
(unclaimed sequence; phosphomevalonate **kinase** genes from plants identified by sequence homol. and their use in screening for herbicides)

L3 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1993:599841 CAPLUS
DN 119:199841
TI Plant carrying genes coding for enzymes of the phytosterol biosynthesis pathway and process for the production of same
IN Lejeune, Fabienne; Tourte, Monique; Oulmouden, Ahmad; Karst, Francis
PA Verneuil Recherche, Fr.
SO PCT Int. Appl., 75 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9316187	A1	19930819	WO 1993-FR134	19930209
	W: CA, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	FR 2687284	A1	19930820	FR 1992-1712	19920214
	FR 2687284	B1	19950623		
	EP 626014	A1	19941130	EP 1993-905378	19930209
	EP 626014	B1	20011024		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	AT 207540	E	20011115	AT 1993-905378	19930209
PRAI	FR 1992-1712	A	19920214		
	WO 1993-FR134	W	19930209		

AB Plants with more rapid development, enhanced productivity, or enhanced regenerative ability contain heterologous genes encoding early steps in the phytosterol biosynthetic pathway, or contain more copies or mutant copies of the endogenous genes. The preferred plants are cruciferous plants such as rape, sunflower, eggplant, and soybean. The transgenic plants may express the mevalonate **kinase** gene (e.g. gene ERG12), the farnesyl diphosphate synthetase gene (e.g. gene ERG20), or the mevalonyl 5-phosphate **kinase** gene (e.g. gene **ERG8**). Transfer vector pFAB2, contg. yeast gene ERG12, was introduced into tobacco with A. tumefaciens. Transgenic tobacco prepd. from the transformed cells exhibited enhanced metabolic **activities**, 2.5-3-fold elevated chlorophyll levels, and 1.6-2-fold elevated gaseous exchange rate relative to unaltered tobacco plants.

AB Plants with more rapid development, enhanced productivity, or enhanced regenerative ability contain heterologous genes encoding early steps in the phytosterol biosynthetic pathway, or contain more copies or mutant copies of the endogenous genes. The preferred plants are cruciferous plants such as rape, sunflower, eggplant, and soybean. The transgenic plants may express the mevalonate **kinase** gene (e.g. gene ERG12), the farnesyl diphosphate synthetase gene (e.g. gene ERG20), or the mevalonyl 5-phosphate **kinase** gene (e.g. gene **ERG8**). Transfer vector pFAB2, contg. yeast gene ERG12, was introduced into tobacco with A. tumefaciens. Transgenic tobacco prepd. from the transformed cells exhibited enhanced metabolic **activities**, 2.5-3-fold elevated chlorophyll levels, and 1.6-2-fold elevated gaseous exchange rate relative to unaltered tobacco plants.

ST plant transgenic phytosterol biosynthesis enzyme gene; mevalonate **kinase** gene ERG12 transgenic plant; farnesyl diphosphate synthase gene transgenic plant; mevalonyl phosphate **kinase** gene transgenic plant

IT Plasmid and Episome
(pFAB2, mevalonate **kinase** gene ERG12 of yeast on, expression in tobacco of)
IT Gene, microbial
RL: BIOL (Biological study)
(**ERG8**, for mevalonyl 5-phosphate **kinase** of yeast, transgenic plant contg. heterologous or mutated or increased copy no)
IT Gene, microbial
RL: BIOL (Biological study)
(ERG12, for mevalonate **kinase** of yeast, transgenic plant contg. heterologous or mutated or increased copy no)
IT 9026-46-4 9026-52-2, Mevalonate **kinase** 50812-36-7, Farnesyl diphosphate synthetase
RL: BIOL (Biological study)
(gene for, transgenic plant contg. heterologous or mutated or increased copy no.)

L3 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 1
AN 91117228 MEDLINE
DN 91117228 PubMed ID: 1846667
TI Cloning and characterization of **ERG8**, an essential gene of Saccharomyces cerevisiae that encodes phosphomevalonate **kinase**.
IDS
AU Tsay Y H; Robinson G W
CS Department of Cellular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543.
SO MOLECULAR AND CELLULAR BIOLOGY, (1991 Feb) 11 (2) 620-31.
Journal code: 8109087. ISSN: 0270-7306.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-M63648; GENBANK-M64559; GENBANK-M64560; GENBANK-M64561; GENBANK-M64562; GENBANK-M64563; GENBANK-M64564; GENBANK-M64565; GENBANK-M64566; GENBANK-X59842
EM 199103
ED Entered STN: 19910329
Last Updated on STN: 19970203
Entered Medline: 19910304
AB Saccharomyces cerevisiae strains that contain the ery8-1 mutation are temperature sensitive for growth due to a defect in phosphomevalonate **kinase**, an enzyme of isoprene and ergosterol biosynthesis. A plasmid bearing the yeast **ERG8** gene was isolated from a YCp50 genomic library by functional complementation of the **erg8**-1 mutant strain. Genetic analysis demonstrated that integrated copies of an **ERG8** plasmid mapped to the **erg8** locus, confirming the identity of this clone. Southern analysis showed that **ERG8** was a single-copy gene. Subcloning and DNA sequencing defined the functional **ERG8** regulon as an 850-bp upstream region and an adjacent 1,272-bp open reading frame. The deduced 424-amino-acid **ERG8** protein showed no homology to known proteins except within a putative ATP-binding domain present in many kinases. Disruption of the chromosomal **ERG8** coding region by integration of URA3 or HIS3 marker fragments was lethal in haploid cells, indicating that this gene is essential. Expression of the **ERG8** gene in S. cerevisiae from the galactose-inducible galactokinase (GAL1) promoter resulted in 1,000-fold-elevated levels of phosphomevalonate **kinase** enzyme **activity**. Overproduction of a soluble protein with the predicted 48-kDa size for phosphomevalonate **kinase** was also observed in the yeast cells.
TI Cloning and characterization of **ERG8**, an essential gene of Saccharomyces cerevisiae that encodes phosphomevalonate **kinase**.
AB Saccharomyces cerevisiae strains that contain the ery8-1 mutation are temperature sensitive for growth due to a defect in phosphomevalonate **kinase**, an enzyme of isoprene and ergosterol biosynthesis. A

plasmid bearing the yeast **ERG8** gene was isolated from a YCp50 genomic library by functional complementation of the **erg8**-1 mutant strain. Genetic analysis demonstrated that integrated copies of an **ERG8** plasmid mapped to the **erg8** locus, confirming the identity of this clone. Southern analysis showed that **ERG8** was a single-copy gene. Subcloning and DNA sequencing defined the functional **ERG8** regulon as an 850-bp upstream region and an adjacent 1,272-bp open reading frame. The deduced 424-amino-acid **ERG8** protein showed no homology to known proteins except within a putative ATP-binding domain present in many kinases. Disruption of the chromosomal **ERG8** coding region by integration of URA3 or HIS3 marker fragments was lethal in haploid cells, indicating that this gene is essential. Expression of the **ERG8** gene in S. cerevisiae from the galactose-inducible galactokinase (GAL1) promoter resulted in 1,000-fold-elevated levels of phosphomevalonate **kinase** enzyme **activity**. Overproduction of a soluble protein with the predicted 48-kDa size for phosphomevalonate **kinase** was also observed in the yeast cells.

CN 0 (DNA, Fungal); 0 (Plasmids); 0 (Recombinant Proteins); EC 2.7 (Phosphotransferases); EC 2.7.4.2 (phosphomevalonate **kinase**)

GEN **erg8**

=>

09/988863

End of Result Set

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L1: Entry 7 of 7 File: DWPI Feb 25, 2003

DERWENT-ACC-NO: 2001-218441
DERWENT-WEEK: 200317

TITLE: New polypeptides and polynucleotides (ERG8) from Candida albicans, useful in assays for identifying inhibitors of phosphomevalonate kinase activity and as reagents for diagnosing C. albicans infection

INVENTOR: ROSAMOND, J D C; SCHNELL, N F

PATENT-ASSIGNEE: ASTRAZENECA AB (ASTR), ASTRAZENECA UK LTD (ASTR)

PRIORITY-DATA: 1999GB-0019766 (August 21, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 2003507060 W	February 25, 2003		041	C12N015/09
WO 200114533 A2	March 1, 2001	E	029	C12N009/00
EP 1212431 A2	June 12, 2002	E	000	C12N015/54

DESIGNATED-STATES: JP MG US AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
JP2003507060W	August 15, 2000	2000WO-GB03100	
JP2003507060W	August 15, 2000	2001JP-0518847	
JP2003507060W		WO 200114533	Based on
WO 200114533A2	August 15, 2000	2000WO-GB03100	
EP 1212431A2	August 15, 2000	2000EP-0951744	
EP 1212431A2	August 15, 2000	2000WO-GB03100	
EP 1212431A2		WO 200114533	Based on

INT-CL (IPC): C07 K 16/40; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/00; C12 N 9/12; C12 N 15/09; C12 N 15/54; C12 P 21/08; C12 Q 1/68; G01 N 33/50; G01 N 33/53; G01 N 33/566; C12 N 9/12; C12 Q 1/68; C12 R 1:725; C12 R 1:725

ABSTRACTED-PUB-NO: WO 200114533A
BASIC-ABSTRACT:

NOVELTY - A purified polypeptide referred to as ERG8 comprising the sequence having 432 amino acids (I) (derived from Candida albicans) fully defined in the specification, a sequence possessing at least 80% identity to (I), or an isolated polypeptide of at least 15 contiguous amino acids of the polypeptide above, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an antibody specific for the polypeptide;

(2) a purified polynucleotide (ERG8 gene) comprising:

(a) a nucleotide sequence encoding the polypeptide or a sequence possessing at least 80% identity to it; or

(b) a polynucleotide of at least 15 nucleotides in length, which is capable of specifically hybridizing to a DNA sequence having 547, 577, 1763 or 1299 base pairs (bp) fully defined in the specification, or the complement of these DNA sequences;

(3) an expression vector comprising the polynucleotide;

(4) a host cell containing the expression vector;

(5) a method (M1) for producing the polypeptide;

(6) a method (M2) for identifying compounds that modulate the activity of phosphomevalonate kinase (PMK) comprising:

(a) contacting a test compound with the polypeptide; and

(b) determining the effect that the test compound has on the activity of the polypeptide;

(7) a compound identified by (M2);

(8) a method (M3) for detecting or diagnosing the presence of C. albicans in a test sample comprising contacting the sample with an agent capable of detecting the polypeptide or a sequence possessing at least 80% similarity to it, or a nucleic acid sequence encoding the polypeptide or a sequence possessing at least 80% identity to it; and

(9) a diagnostic kit for detecting the presence of C. albicans comprising one or more diagnostic probes and/or diagnostic primers and/or antibodies capable of selectively hybridizing or binding to the polynucleotide or polypeptide .

USE - The polypeptide (ERG8) is useful in an assay for identifying compounds that inhibit phosphomevalonate kinase (PMK) activity (claimed). These inhibitors are useful as anti-fungal agents. The polynucleotides and polypeptides are also useful as diagnostic reagents for diagnosing C. albicans infection.

ABSTRACTED-PUB-NO: WO 200114533A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/1

DERWENT-CLASS: B04 D16
CPI-CODES: B04-C01G; B04-E03F; B04-E05; B04-E08; B04-F0100E; B04-F09; B04-G01; B04-G21; B04-M01; B04-N03A; B11-C07; B12-K04; B14-A04; D05-H05; D05-H11A; D05-H12A; D05-H12E; D05-H14; D05-H17A6; D05-H18B;

09/988863

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=> s Meissner.au.
L1 0 MEISSNER.AU.

=> s kunze.au.
L2 0 KUNZE.AU.

=> s phosphomevalonate kinase or ERG8
L3 247 PHOSPHOMEVALONATE KINASE OR ERG8

=> s l3 and plant#
L4 40 L3 AND PLANT#

=> s l4 and (herbicid## or antibod### or modulator# or affect### activit###)
L5 5 L4 AND (HERBICID## OR ANTIBOD### OR MODULATOR# OR AFFECT### ACTIVIT###)

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 5 DUP REM L5 (0 DUPLICATES REMOVED)

=> d l6 1-5 bib ab kwic

L6 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:123201 CAPLUS
DN 136:162385
TI Methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**
IN Boronat, Albert; Campos, Narciso; Rodriguez-Concepcion, Manuel; Rohmer, Michel; Seeman, Myriam; Valentin, Henry E.; Venkatesh, Tyamagondlu V.; Venkatramesh, Mylavarapu
PA Monsanto Technology, LLC, USA
SO PCT Int. Appl., 155 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002012478	A2	20020214	WO 2001-US24335	20010806
	WO 2002012478	C1	20020704		
	WO 2002012478	A3	20030703		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU	2001090522	A5	20020218	AU 2001-90522	20010806
US	2002069426	A1	20020606	US 2001-921992	20010806
PRAI US	2000-223483P	P	20000807		
WO	2001-US24335	W	20010806		

AB The present invention provides and includes nucleic acids, proteins and **antibodies** assocd. with novel genes in the methyl-D-erythritol phosphate (MEP) biosynthesis pathway. Specifically, a homolog of the Escherichia coli gcpE gene is found in Arabidopsis thaliana which catalyzes the conversion of 2-C-methyl-D-erythritol 2,4-cyclodiphophate to (E)-1-(4-hydroxy-3-methylbut-2-enyl) diphosphate. Partial gene sequences are also provided from soybean, tomato, Mesembryanthemum crystallinum, rice, maize, loblolly pine, soybean, Brassica, and Physcomitrella patens. The invention further encompasses methods utilizing such mols., for example in gene isolation, gene anal. and the prodn. of transgenic **plants**. The present invention also includes transgenic **plants** modified to express proteins assocd. with the MEP pathway. Modulation of isoprenoid, tocopherol, monoterpene, and carotenoid levels can be achieved in transgenic **plants**.

TI Methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**

AB The present invention provides and includes nucleic acids, proteins and **antibodies** assocd. with novel genes in the methyl-D-erythritol phosphate (MEP) biosynthesis pathway. Specifically, a homolog of the Escherichia coli gcpE gene is found in Arabidopsis thaliana which catalyzes the conversion of 2-C-methyl-D-erythritol 2,4-cyclodiphophate to (E)-1-(4-hydroxy-3-methylbut-2-enyl) diphosphate. Partial gene sequences are also provided from soybean, tomato, Mesembryanthemum crystallinum, rice, maize, loblolly pine, soybean, Brassica, and Physcomitrella patens. The invention further encompasses methods utilizing such mols., for example in gene isolation, gene anal. and the prodn. of transgenic **plants**. The present invention also includes transgenic **plants** modified to express proteins assocd. with the MEP pathway. Modulation of isoprenoid, tocopherol, monoterpene, and carotenoid levels can be achieved in transgenic **plants**.

ST methylerythritol phosphate pathway gene gcpE sequence **plant**; Arabidopsis methylerythritol phosphate pathway gene gcpE; rice methylerythritol phosphate pathway gene gcpE; isoprenoid modulation gene gcpE methylerythritol phosphate pathway

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ACP (acyl-carrier), use of gene promoter in genetic constructs; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Gene, microbial
Gene, **plant**
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(gcpE; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Agrobacterium tumefaciens
(genetic vector for **plant** transformation; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Animal cell
(insect, transgenic; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Animal cell
(mammalian, transgenic; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Flours and Meals
(manuf. of; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Fats and Glyceridic oils, preparation
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(manuf. of; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Arabidopsis thaliana
Corn
DNA sequences
Escherichia coli
Mesembryanthemum crystallinum
Molecular cloning
Physcomitrella patens
Pine (Pinus taeda)
Protein sequences
Rice (Oryza sativa)
Soybean (Glycine max)
Tomato
Transformation, genetic
cDNA sequences
(methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT **Antibodies**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Promoter (genetic element)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Transit peptides
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Carotenes, biological studies
Gibberellins
Isoprenoids
Monoterpenes
Terpenes, biological studies
Tocopherols
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(modulation of levels of; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Albumins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(napins, use of gene promoter in genetic constructs; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(oleosins, use of gene promoter in genetic constructs; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)
IT Carotenes, biological studies
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)

(oxy, modulation of levels of; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Globulins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(phaseolins, use of gene promoter in genetic constructs; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Bacteria (Eubacteria)
Brassica campestris
Brassica napus
Canola
Castor bean
Coconut (Cocos nucifera)
Cotton
Crambe
Embryophyta
Flaxseed
Fruit
Fungi
Mustard (Brassica)
Oil palm (Elaeis)
Peanut (Arachis hypogaea)
Plant cell
Rapeseed
Safflower (Carthamus tinctorius)
Seed
Sesame (Sesamum indicum)
Sunflower
Wheat
(transgenic; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Zeins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(use of gene promoter in genetic constructs; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Conglycinins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(use of subunit a' gene promoter in genetic constructs; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Gene, **plant**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(yfgA; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT Gene, **plant**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(yfgB; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 397436-85-0, Enzyme (Oryza sativa gene gcpE) 397437-30-8, Enzyme (Arabidopsis thaliana gene gcpE) 397437-31-9, Enzyme (Oryza sativa gene gcpE) 397437-32-0, Enzyme (Escherichia coli gene gcpE)
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 151435-51-7 206440-72-4, 2-C-Methyl-D-erythritol 4-phosphate 396726-03-7
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 9024-66-2, Diphosphomevalonate decarboxylase 9026-46-4, 5-**Phosphomevalonate kinase** 9026-52-2, Mevalonate kinase 9033-27-6, IPP isomerase 210756-42-6, 1-Deoxy-D-xylulose 5-phosphate

reductoisomerase 251990-59-7, 4-Diphosphocytidyl-2-C-methylerythritol synthase 287480-92-6, 2-C-Methyl-D-erythritol 2,4-cyclodiphosphate synthase
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 398144-56-4, 1-Hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 358-71-4P, Isopentenyl diphosphate 358-72-5P 6829-55-6P, Tocotrienol
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(modulation of levels of; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 397436-83-8 397436-84-9, DNA (Oryza sativa gene gcpE plus flanks)
397436-86-1 397436-87-2, DNA (Escherichia coli gene gcpE) 397436-88-3
397436-89-4 397436-90-7 397436-91-8 397436-92-9 397436-93-0
397436-94-1 397436-95-2 397436-96-3 397436-97-4 397436-98-5
397436-99-6 397437-00-2 397437-01-3 397437-02-4 397437-03-5
397437-04-6 397437-05-7 397437-06-8 397437-07-9 397437-08-0
397437-09-1 397437-10-4 397437-11-5 397437-12-6 397437-13-7
397437-14-8 397437-15-9 397437-16-0 397437-17-1 397437-18-2
397437-19-3 397437-20-6 397437-21-7 397437-22-8 397437-23-9
397437-24-0 397437-25-1 397437-26-2 397437-27-3 397437-28-4
397437-29-5
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 397440-71-0 397440-72-1 397440-73-2 397440-74-3 397440-75-4
397440-76-5 397440-77-6 397440-78-7 397440-79-8 397440-80-1
397440-81-2 397440-82-3 397440-83-4 397440-84-5 397440-85-6
397440-86-7 397440-87-8 397440-88-9 397440-89-0 397440-90-3
397440-91-4 397440-92-5 397440-93-6 397440-94-7 397440-95-8
397440-96-9 397440-97-0 397440-98-1 397441-00-8 397441-01-9
RL: PRP (Properties)
(unclaimed nucleotide sequence; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 397440-99-2
RL: PRP (Properties)
(unclaimed protein sequence; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 253167-42-9 397311-78-3
RL: PRP (Properties)
(unclaimed sequence; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

IT 9078-38-0, Soybean trypsin inhibitor 37256-86-3, Stearoyl-ACP desaturase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(use of gene promoter in genetic constructs; methyl-D-erythritol phosphate pathway gene gcpE from Arabidopsis thaliana and other **plants**)

L6 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:107554 CAPLUS
DN 136:164278
TI Manipulation of genes for enzymes of the mevalonate and isoprenoid biosynthesis to create novel traits in transgenic organisms
IN Hahn, Frederick M.; Kuehnle, Adelheid R.
PA USA
SO PCT Int. Appl., 193 pp.
CODEN: PIXXD2

DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002010398	A2	20020207	WO 2001-US24037	20010731
	WO 2002010398	A3	20030626		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

	US 2003033626	A1	20030213	US 2001-918740	20010731
PRAI	US 2000-221703P	P	20000731		

AB Disclosed are the uses of specific genes of the mevalonate and isoprenoid biosynthetic pathways, and of inactive gene sites (the pseudogene) to increase biosynthesis of isopentenyl diphosphate, dimethylallyl diphosphate and isoprenoid pathway derived products in the plastids of transgenic **plants** and microalgae; create novel antibiotic resistant transgenic **plants** and microalgae, and (3) create a novel selection system and/or targeting sites for mediating the insertion of genetic material into **plant** and microalgae plastids. The specific polynucleotides to be used, solely or in any combination thereof, are publicly available from GeneBank and contain open reading frames having sequences that upon expression will produce active proteins with the following enzyme activities: (a) acetoacetyl CoA thiolase (EC 2.3.1.9), (b) 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (EC 4.1.3.5), (c) HMG-CoA reductase (EC 1.1.1.34), (d) mevalonate kinase (EC 2.7.1.36), (e) **phosphomevalonate kinase** (EC 2.7.4.2), (f) mevalonate diphosphate decarboxylase (EC 4.1.1.33), (g) isopentenyl diphosphate (IPP) isomerase (EC 5.3.3.2), and (b) phytoene synthase (EC 2.5.1.32). Methods for cloning of the genes, construction of expression constructs, transformation and selection of microalgae and **plants** are described in detail.

AB Disclosed are the uses of specific genes of the mevalonate and isoprenoid biosynthetic pathways, and of inactive gene sites (the pseudogene) to increase biosynthesis of isopentenyl diphosphate, dimethylallyl diphosphate and isoprenoid pathway derived products in the plastids of transgenic **plants** and microalgae; create novel antibiotic resistant transgenic **plants** and microalgae, and (3) create a novel selection system and/or targeting sites for mediating the insertion of genetic material into **plant** and microalgae plastids. The specific polynucleotides to be used, solely or in any combination thereof, are publicly available from GeneBank and contain open reading frames having sequences that upon expression will produce active proteins with the following enzyme activities: (a) acetoacetyl CoA thiolase (EC 2.3.1.9), (b) 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (EC 4.1.3.5), (c) HMG-CoA reductase (EC 1.1.1.34), (d) mevalonate kinase (EC 2.7.1.36), (e) **phosphomevalonate kinase** (EC 2.7.4.2), (f) mevalonate diphosphate decarboxylase (EC 4.1.1.33), (g) isopentenyl diphosphate (IPP) isomerase (EC 5.3.3.2), and (b) phytoene synthase (EC 2.5.1.32). Methods for cloning of the genes, construction of expression constructs, transformation and selection of microalgae and **plants** are described in detail.

ST isopentenyl pyrophosphate biosynthesis **plant** genetic engineering; **herbicide** resistance mevalonate biosynthesis transgenic **plant**; transplastomic **plant** microalgae isoprenoid biosynthesis; synthetic operon isoprenoid biosynthesis genetic engineering; mevalonate biosynthesis **plant** genetic engineering

IT **Herbicide** resistance

Microalgae
(altering patterns of mevalonate biosynthesis in; manipulation of genes for enzymes of mevalonate and isoprenoid biosynthesis to create novel traits in transgenic organisms)

IT Gene, **plant**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(infA, integration of transforming DNA into pseudogene; manipulation of genes for enzymes of mevalonate and isoprenoid biosynthesis to create novel traits in transgenic organisms)

IT Liliales
Metabolism, **plant**
Petunia
Potato (Solanum tuberosum)
Rosaceae
Solanaceae
Tobacco
Tomato
(manipulation of genes for enzymes of mevalonate and isoprenoid biosynthesis to create novel traits in transgenic organisms)

IT Genetic engineering
(of isoprenoid biosynthesis in **plants** and algae; manipulation of genes for enzymes of mevalonate and isoprenoid biosynthesis to create novel traits in transgenic organisms)

L6 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:658670 CAPLUS
DN 137:197518
TI cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof
IN Falco, Saverio Carl; Famodu, Omolayo O.
PA USA
SO U.S. Pat. Appl. Publ., 39 pp., Cont.-in-part of U.S. Ser. No. 433,242, abandoned.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2002119546	A1	20020829	US 2001-909745	20010720
PRAI US 1998-107241P	P	19981105		
US 1999-433242	B2	19991104		

AB This invention relates to an isolated nucleic acid fragment encoding squalene biosynthetic enzymes, in particular, mevalonate kinase (claimed) and **phosphomevalonate kinase** (not claimed) from corn, rice, soybean, and wheat. The invention also relates to the construction of a chimeric gene encoding all or a portion of the above enzymes, in sense or antisense orientation, wherein expression of the chimeric gene results in prodn. of their altered levels in a transformed host cell.

TI cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof
AB This invention relates to an isolated nucleic acid fragment encoding squalene biosynthetic enzymes, in particular, mevalonate kinase (claimed) and **phosphomevalonate kinase** (not claimed) from corn, rice, soybean, and wheat. The invention also relates to the construction of a chimeric gene encoding all or a portion of the above enzymes, in sense or antisense orientation, wherein expression of the chimeric gene results in prodn. of their altered levels in a transformed host cell.

ST squalene biosynthesis mevalonate kinase cDNA sequence corp **plant** ; **phosphomevalonate kinase** cDNA sequence corp **plant** squalene biosynthesis

IT Corn

Genetic engineering
Molecular cloning
Protein sequences
Rice (Oryza sativa)
Soybean (Glycine max)
Wheat
cDNA sequences
(cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT Viral vectors
(for mevalonate kinase expressing; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT Drug screening
(for mevalonate kinase **modulators**; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT Gene, **plant**
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(for mevalonate kinase or **phosphomevalonate kinase**, of corp **plant**; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT Bacteria (Eubacteria)
Liliopsida
Magnoliopsida
Plant cell
Yeast
(host; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT Embryophyta
(transgenic, expressing mevalonate kinase; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT 453622-10-1P 453622-11-2P 453622-12-3P 453622-13-4P 453622-14-5P 453622-15-6P 453622-16-7P 453622-17-8P
RL: AGR (Agricultural use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT 9026-46-4P, Kinase (phosphorylating), phosphomevalonate 9026-52-2P, Kinase (phosphorylating), mevalonate
RL: AGR (Agricultural use); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)
(cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT 111-02-4, Squalene
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(enzymes for the biosynthesis of; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

IT 453622-02-1 453622-03-2 453622-04-3 453622-05-4 453622-06-5 453622-07-6 453622-08-7 453622-09-8
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from

corp **plant** and use thereof)
IT 453640-30-7 453640-32-9 453640-33-0 453640-35-2
RL: PRP (Properties)
(unclaimed nucleotide sequence; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)
IT 453640-31-8 453640-34-1 453640-36-3 453640-37-4 453640-39-6
RL: PRP (Properties)
(unclaimed protein sequence; cDNA for squalene biosynthetic enzymes - mevalonate kinase and **phosphomevalonate kinase** from corp **plant** and use thereof)

L6 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:391285 CAPLUS
DN 136:381391
TI **Phosphomevalonate kinase** genes from **plants** identified by sequence homology and their use in screening for **herbicides**
IN Meissner, Ruth; Lechelt-Kunze, Christa
PA Bayer AG, Germany
SO Ger. Offen., 18 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 10057755	A1	20020523	DE 2000-10057755	20001122
EP 1209236	A1	20020529	EP 2001-126453	20011109
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2002355067	A2	20021210	JP 2001-350270	20011115
US 2002123427	A1	20020905	US 2001-988863	20011121
PRAI DE 2000-10057755	A	20001122		

AB **Plant** genes showing sequence homol. to the **phosphomevalonate kinase** gene **ERG8** of Saccharomyces cerevisiae are identified for use in the development of **herbicides** acting on isoprenoid biosynthesis. The Arabidopsis thaliana **phosphomevalonate kinase** gene was identified by suppression subtractive hybridization.
TI **Phosphomevalonate kinase** genes from **plants** identified by sequence homology and their use in screening for **herbicides**
AB **Plant** genes showing sequence homol. to the **phosphomevalonate kinase** gene **ERG8** of Saccharomyces cerevisiae are identified for use in the development of **herbicides** acting on isoprenoid biosynthesis. The Arabidopsis thaliana **phosphomevalonate kinase** gene was identified by suppression subtractive hybridization.
ST **phosphomevalonate kinase** gene discovery **plant** **herbicide** development
IT Protein sequences
(for **phosphomevalonate kinase** sequence homolog of Arabidopsis; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)
IT cDNA sequences
(for **phosphomevalonate kinase** sequence homologs of **plants**; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)
IT Gene, **plant**
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)

(for **phosphomevalonate kinase**, identification by sequence homol. of; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT Genetic methods
(gene discovery; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT Hormones, **plant**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**phosphomevalonate kinase** and biosynthesis and activity of; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT **Herbicides**
Molecular cloning
(**phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT Arabidopsis thaliana
Cotton
Medicago truncatula
Pine (Pinus radiata)
(**phosphomevalonate kinase** sequence homolog of; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT **Antibodies**
RL: AGR (Agricultural use); ARG (Analytical reagent use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(to **phosphomevalonate kinase** sequence homologs; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT 427909-05-5
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT 199693-91-9, GenBank AA660847 234641-71-5, GenBank AI727861
427909-04-4 427909-06-6
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT 9026-46-4, **Phosphomevalonate kinase**
RL: AGR (Agricultural use); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(sequence homologs; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

IT 427909-20-4
RL: PRP (Properties)
(unclaimed sequence; **phosphomevalonate kinase** genes from **plants** identified by sequence homol. and their use in screening for **herbicides**)

L6 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2001:618140 CAPLUS
DN 135:191325
TI Gene disruption methodologies for identification of drug targets in diploid pathogens, particularly Candida albicans

IN Roemer, Terry; Jiang, Bo; Boone, Charles; Bussey, Howard
PA Elitra Pharmaceuticals, Inc., USA
SO PCT Int. Appl., 324 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001060975	A2	20010823	WO 2001-US5551	20010220
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1292668	A2	20030319	EP 2001-916144	20010220
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRAI US 2000-183534P	P	20000218		
WO 2001-US5551	W	20010220		

AB The present invention provides methods and compns. that enable the exptl. detn. as to whether any gene in the genome of a diploid pathogenic organism is essential, and whether it is required for virulence or pathogenicity. The methods involve the construction of genetic mutants in which one allele of a specific gene is inactivated while the other allele of the gene is placed under conditional expression. The identification of essential genes and those genes crit. to the development of virulent infections, provides a basis for the development of screens for new drugs against such pathogenic organisms. The present invention further provides Candida albicans genes that are demonstrated to be essential and are potential targets for drug screening. The nucleotide sequence of the target genes can be used for various drug discovery purposes, such as expression of the recombinant protein, hybridization assay and construction of nucleic acid arrays. The uses of proteins encoded by the essential genes, and genetically engineered cells comprising modified alleles of essential genes in various screening methods are also encompassed by the invention. The method for construction of strains is referred to as GRACE (gene replacement and controlled expression) and recombinant Candida albicans strains are called GRACE strains. GRACE involves homologous recombination between transgene cassettes and genomic DNA.

IT **Antibodies**
RL: ARG (Analytical reagent use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(GRACE (gene replacement and controlled expression) and use of gene disruption methodologies for identification of drug targets in diploid pathogens, particularly Candida albicans)

IT Bird (Aves)
Mammal (Mammalia)
Plant (Embryophyta)
Vertebrate (Vertebrata)
(infection by Candida albicans; gene disruption methodologies for identification of drug targets in diploid pathogens, particularly Candida albicans)

IT 188449-97-0 198424-31-6, Protein (Candida albicans gene CaRho-1) 328054-48-4 356819-93-7, Protein (Candida albicans gene SAT2) 356819-94-8, Protein (Candida albicans gene POP7) 356819-95-9, Protein (Candida albicans gene ALG7) 356819-96-0, Protein (Candida albicans gene RRP7) 356819-97-1 356819-98-2, Protein (Candida albicans gene SAS10) 356819-99-3, Protein (Candida albicans gene DBF4) 356820-00-3, Protein

(Candida albicans gene APC4) 356820-01-4 356820-02-5 356820-03-6, Protein (Candida albicans gene CHO1) 356820-04-7 356820-05-8 356820-06-9, Protein (Candida albicans gene ERG11) 356820-07-0 356820-08-1, Protein (Candida albicans gene MED6) 356820-09-2, Protein (Candida albicans gene ORC6) 356820-10-5, Protein (Candida albicans gene SPC97) 356820-11-6 356820-12-7, Protein (Candida albicans gene STS1) 356820-13-8 356820-14-9, Protein (Candida albicans gene DPB11) 356820-15-0 356820-16-1, Protein (Candida albicans gene NNF1) 356820-17-2, Protein (Candida albicans gene AUR1) 356820-18-3 356820-19-4, Protein (Candida albicans gene RPC37) 356820-20-7, Protein (Candida albicans gene LAS1) 356820-21-8 356820-22-9 356820-23-0 356820-24-1, Protein (Candida albicans gene SFI1) 356820-25-2 356820-26-3, Protein (Candida albicans gene CDC45) 356820-27-4, Protein (Candida albicans gene FKS1) 356820-28-5, Protein (Candida albicans gene ILV5) 356820-29-6, Protein (Candida albicans gene YML6) 356820-30-9, Protein (Candida albicans gene SWP1) 356820-31-0, Protein (Candida albicans gene ROT1) 356820-32-1, Protein (Candida albicans gene **ERG8**) 356820-33-2, Protein (Candida albicans gene RCP1) 356820-34-3 356820-35-4 356820-36-5, Protein (Candida albicans gene RPC31) 356820-37-6 356820-38-7, Protein (Candida albicans gene CSL4) 356820-39-8 356820-40-1 356820-41-2 356820-42-3 356820-43-4, Protein (Candida albicans gene UFE1) 356820-44-5, Protein (Candida albicans gene SPP2) 356820-45-6 356820-46-7 356820-47-8, Protein (Candida albicans gene TBF1) 356820-48-9, Protein (Candida albicans gene CDC60) 356820-49-0, Protein (Candida albicans gene CET1) 356820-50-3, Protein (Candida albicans gene DPB2)
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(amino acid sequence; gene disruption methodologies for identification of drug targets in diploid pathogens, particularly Candida albicans)

IT 356819-31-3, DNA (Candida albicans gene SAT2) 356819-32-4, DNA (Candida albicans gene POP7) 356819-33-5, DNA (Candida albicans gene ALG7) 356819-34-6, DNA (Candida albicans gene RRP7) 356819-35-7 356819-36-8, DNA (Candida albicans gene SAS10) 356819-37-9, DNA (Candida albicans gene DBF4) 356819-38-0, DNA (Candida albicans gene APC4) 356819-39-1 356819-40-4 356819-41-5, DNA (Candida albicans gene SEC20) 356819-42-6, DNA (Candida albicans gene CHO1) 356819-43-7 356819-44-8 356819-45-9, DNA (Candida albicans gene ERG11) 356819-46-0 356819-47-1, DNA (Candida albicans gene MED6) 356819-48-2, DNA (Candida albicans gene ORC6) 356819-49-3, DNA (Candida albicans gene SPC97) 356819-50-6 356819-51-7, DNA (Candida albicans gene STS1) 356819-52-8 356819-53-9, DNA (Candida albicans gene DPB11) 356819-54-0 356819-55-1, DNA (Candida albicans gene NNF1) 356819-56-2, DNA (Candida albicans gene AUR1) 356819-57-3 356819-58-4, DNA (Candida albicans gene RPC37) 356819-59-5, DNA (Candida albicans gene LAS1) 356819-60-8 356819-61-9 356819-62-0 356819-63-1, DNA (Candida albicans gene SFI1) 356819-64-2 356819-65-3, DNA (Candida albicans gene CDC45) 356819-66-4, DNA (Candida albicans gene FKS1) 356819-67-5, DNA (Candida albicans gene ILV5) 356819-68-6, DNA (Candida albicans gene YML6) 356819-69-7, DNA (Candida albicans gene TUB1) 356819-70-0, DNA (Candida albicans gene SWP1) 356819-71-1, DNA (Candida albicans gene ROT1) 356819-72-2, DNA (Candida albicans gene **ERG8**) 356819-73-3, DNA (Candida albicans gene FCP1) 356819-74-4 356819-75-5 356819-76-6, DNA (Candida albicans gene RPC31) 356819-77-7 356819-78-8, DNA (Candida albicans gene CSL4) 356819-79-9 356819-80-2 356819-81-3 356819-82-4 356819-83-5, DNA (Candida albicans gene UFE1) 356819-84-6, DNA (Candida albicans gene SPP2) 356819-85-7 356819-86-8 356819-87-9, DNA (Candida albicans gene TBF1) 356819-88-0 356819-89-1, DNA (Candida albicans gene CET1) 356819-90-4, DNA (Candida albicans gene RHO1) 356819-91-5, DNA (Candida albicans gene DPB2) 356819-92-6, DNA (Candida albicans gene CDC60)
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(nucleotide sequence; gene disruption methodologies for identification

of drug targets in diploid pathogens, particularly Candida albicans)

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L11 ANSWER 2 OF 2 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
AN 1999415069 EMBASE
TI Isopentenyl diphosphate biosynthesis via a mevalonate-independent pathway: Isopentenyl monophosphate kinase catalyzes the terminal enzymatic step.
AU Lange B.M.; Croteau R.
CS R. Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6430, United States. croteau@mail.wsu.edu
SO Proceedings of the National Academy of Sciences of the United States of America, (23 Nov 1999) 96/24 (13714-13719).
Refs: 36
ISSN: 0027-8424 CODEN: PNASA6
CY United States
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB In plants, the biosynthesis of isopentenyl diphosphate, the central precursor of all isoprenoids, proceeds via two separate pathways. The cytosolic compartment harbors the mevalonate pathway, whereas the newly discovered deoxyxylulose 5-phosphate pathway, which also operates in certain eubacteria, including Escherichia coli, is localized to plastids. Only the first two steps of the plastidial pathway, which involve the condensation of pyruvate and glyceraldehyde 3-phosphate to deoxyxylulose 5-phosphate followed by intramolecular rearrangement and reduction to 2-C-methylerythritol 4- phosphate, have been established. Here we report the cloning from peppermint (Mentha x piperita) and E. coli, and expression, of a kinase that catalyzes the phosphorylation of isopentenyl monophosphate as the last step of this biosynthetic sequence to isopentenyl diphosphate. The plant gene defines an ORF of 1,218 bp that, when the proposed plastidial targeting sequence is excluded, corresponds to .simeq.308 aa with a mature size of .simeq.33 kDa. The E. coli gene (ychB), which is located at 27.2 min of the chromosomal map, consists of 852 nt, encoding a deduced enzyme of 283 aa with a size of 31 kDa. These enzymes represent a conserved class of the GHMP family of kinases, which includes galactokinase, homoserine kinase, mevalonate kinase, and **phosphomevalonate** kinase, with homologues in **plants** and several eubacteria. Besides the preferred substrate isopentenyl monophosphate, the recombinant peppermint and E. coli kinases also phosphorylate isopentenol, and, much less efficiently, dimethylallyl alcohol, but dimethylallyl monophosphate does not serve as a substrate. Incubation of secretory cells isolated from peppermint glandular trichomes with isopentenyl monophosphate resulted in the rapid production of monoterpenes and sesquiterpenes, confirming that isopentenyl monophosphate is the physiologically relevant, terminal intermediate of the deoxyxylulose 5-phosphate pathway.
AB . . . These enzymes represent a conserved class of the GHMP family of kinases, which includes galactokinase, homoserine kinase, mevalonate kinase, and **phosphomevalonate** kinase, with homologues in **plants** and several eubacteria. Besides the preferred substrate isopentenyl monophosphate, the recombinant peppermint and E. coli kinases also phosphorylate isopentenol, and,. . .
CT Medical Descriptors:
*biosynthesis
*catalysis
plant
plastid
reaction analysis
molecular cloning
escherichia coli
phosphorylation
chromosome map
enzyme activity
article
priority journal

*isoprenoid
*pyrophosphate
*mevalonic acid
*phosphotransferase
pyruvic acid
glyceraldehyde 3 phosphate
xylulose
peppermint
terpene

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